EGG LAYING IN THE ACOELOUS TURBELLARIAN POLYCHOERUS CARMELENSIS[1]

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The few accounts of egg laying in the Turbellaria Acoela are brief and unsatisfactory. The Acoela have one or two genital pores, except Nemertoderma (Steinböck, 1931). The absence of oviducts in the Acoela (Bresslau, 1933) leaves the question of egg emergence unsolved.

Von Graff (1905) concluded that the eggs of *Otocelis rubropunctata, Polychoerus caudatus* and *Convoluta roscoffensis* are liberated through the genital pore, which he considered the normal method for most Acoela. For species which possess no oviducts he agreed with Weldon, Sabussow and Monticelli that the mouth might serve in egg deposition. Yet von Graff pointed out (p. 1960) that oviducts had been described with reasonable certainty for only *Otocelis rubropunctata* and *Polychoerus caudatus* and that *Amphiscolops langerhansi* possesses no formed passage.

The evidence supporting von Graff's conclusions as to the rôle of oviducts and genital aperture was extremely meager. In his account of egg deposition in *Otocelis rubropunctata* (1904) he does not mention the path of emergence of the eggs. Gamble and Keeble (1903) stated that in *Convoluta roscoffensis* " frequently the body breaks in two across the opening of the oviduct . . . ," i.e., presumably at the level of the genital pore. Gardiner (1895) is the only observer who has definitely stated that he witnessed the emergence of the eggs from the genital pore. He undoubtedly considered that the eggs of *Polychoerus caudatus* reach the female pore through the oviducts described and figured by Verrill (1893) and later by himself (1898). In these accounts the term oviduct was substituted for vitellarium, and the vitellaria were figured as uniting into a common duct leading directly to the female aperture. Mark (1892) found no connection between the vitellaria and the genital pore, and Löhner (1910) showed conclusively that this species possesses no oviducts. Löhner considered it probable that the eggs pass through a series of vacuoles in the marginal parenchyma and out by way of the female aperture.

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Subsequent references to egg emergence in the Acoela are equally inconclusive. Von Graff (1911) concluded with reference to *Anaperus gardineri* that the eggs probably leave by way of the mouth or through breaks in the body wall. With regard to the entire group Bresslau (1933, p. 118) stated that the eggs probably pass through " einfache Gewebslücken in das Zentralparenchym, um dann durch die Mundöffnung nach aussen befördert zu werden. Gelengentlich vollzieht sich ihr Austritt noch direkter; durch Ruptur der Körperwand an irgendeiner Stelle." This latter alternative obtains in the case of *Polychoerus carmelensis.*

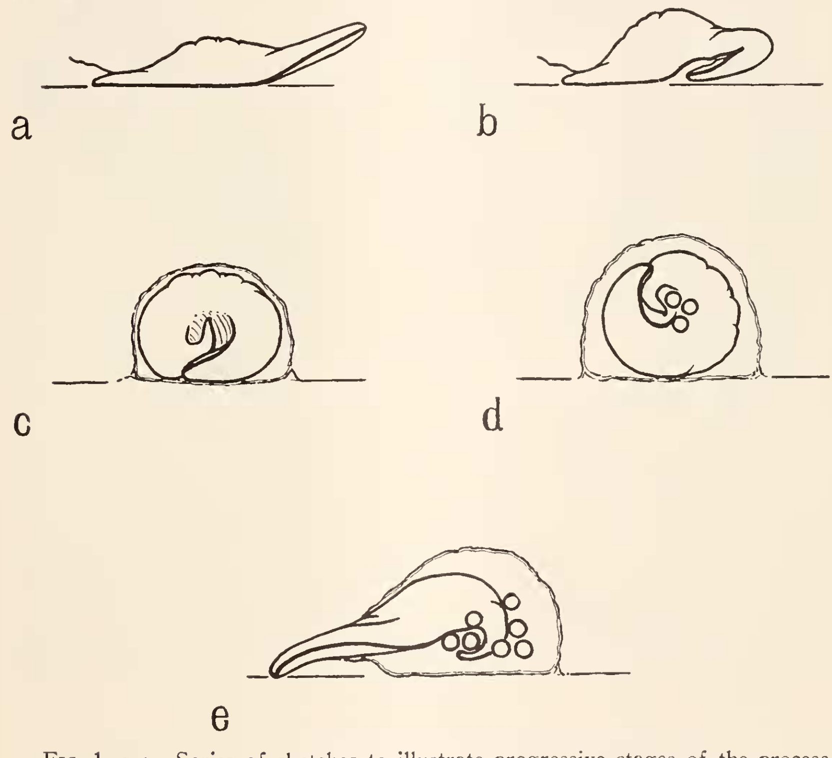


Fig. 1, *a-e*. Series of sketches to illustrate progressive stages of the process of egg laying.

Material and Methods

The reproductive system of *Polychoerus carmelensis* has been described and figured in a recent paper (Costello and Costello, 1938). Specimens about to deposit eggs were observed in situ by means of a hand lens or binocular microscope. Removal for examination invariably terminated the process. Twelve specimens were fixed during egg laying, as follows: five in hot Heath's solution (50° to 60° C.), three in Lillie's, two in Worcester's (one "warm," one at 50° C.), one in Flemming's and one in Champy's solution. The subsequent study of hundreds of specimens has shown that hot fixatives are best for the general preservation of tissues. The specimens were sectioned individually and serially.

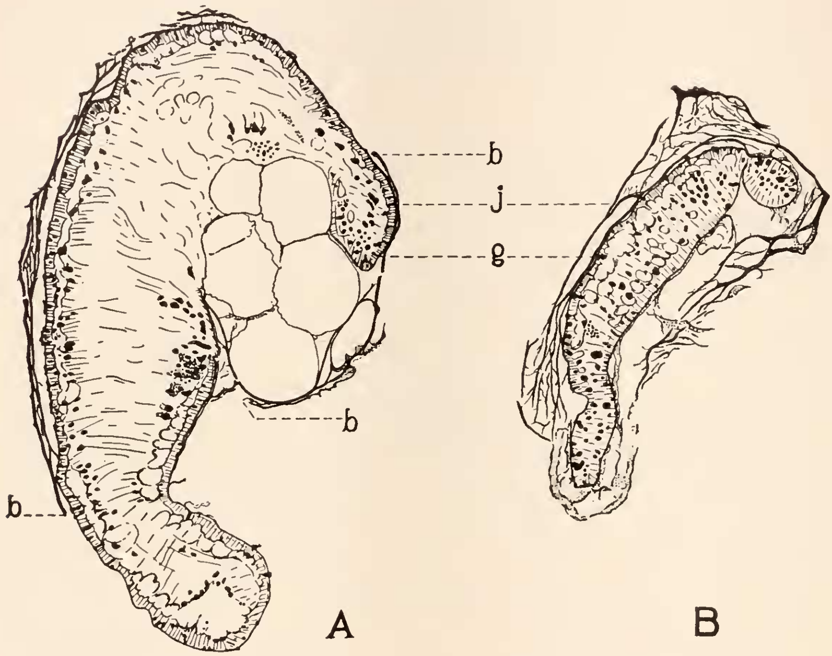


FIG. 2. Oblique sections of an egg-laying individual preserved in Lillie's fixative and stained by the Flemming tricolor method. *A,* through the side of the body; *B,* near the anterior end. To show jelly layers (*j*) of the egg mass, surrounding the worm, and the glands (*g*) which secrete the jelly. In *A,* the jelly is broken at several points (*b*); in *B,* the jelly mass is more complete. Magnification 43 ×.

OBSERVATIONS

An individual about to deposit eggs assumes a resting position on the substratum. The anterior end of the body is at first elevated (Fig. 1*a*), as the animal sways slowly from side to side, and is then bent ventrally in progressive stages (Fig. 1*b*). Meanwhile a jelly-like slime is secreted from the surface of the body and adheres to the substratum. As the anterior portion of the body continues the ventral curling, the posterior end relinquishes its attachment, and curls ventrally under the approaching anterior tip, forming a ball which is attached to the substratum only by the secreted jelly (Fig. 1*c*). During the curling, and subsequently, violent contractions are observable in the region of the vitellaria. The animal then begins to rotate, end over end, rather slowly within the gelatinous mass. During rotation, additional jelly is secreted by the surface of the animal (Fig. 2). Meanwhile, the eggs are emerging from the body of the animal, but are, for the most part, hidden within its folds (Fig. 1*d*). An occasional egg escapes laterally, and may then pass freely over the dorsal surface of the rotating animal (Fig. 5), within the jelly. Rotation continues for three or four minutes, after which the animal unrolls and works its way out of the jelly mass (Fig. 1*e*) through an opening so small as to greatly constrict the body. The eggs are left behind in the central cavity of the jelly mass, which contracts and forces the eggs into close proximity. The entire process of egg laying requires only five or six minutes.

Egg laying occurs infrequently during the early portion of the day, most frequently shortly after sunset (Table I). That egg laying occurs also during the early night is evidenced by large numbers of egg masses found in the morning in late cleavage stages.

The point of emergence of the eggs is concealed by the position assumed by the body during the process. Living individuals forcibly unfolded during egg emergence usually extruded no more eggs. In one case, however, an egg was observed to emerge through the ventral body wall at the level of the bursa.

The fixed material showed very clearly the position occupied by the mature eggs immediately before extrusion, and the point at which the eggs are shed, although no individual egg was fixed in the act of emerging. Of the twelve specimens, one had shed all its mature eggs, three had shed none, the others, a portion.

It has been noted (1938) that the oöcytes move dorsally within the vitellaria as they mature. However, eggs undergoing maturation occupy the greater part of the dorso-ventral thickness of the vitellarium. Immediately before deposition the mature eggs are located medially and ventrally between the body wall and the ventral surface of the bursa. The vitellaria are displaced laterally and the bursa is displaced dorsally, curving around the eggs as shown in Fig. 3. This arrangement was evidenced by the eleven fixed specimens which retained one or more mature eggs.

Evidence as to the point of emergence of the eggs is limited to the nine specimens which had shed one or more eggs at the time of fixation. There was no indication of the emergence of eggs by way of the mouth and intestinal parenchyma, nor by way of the vagina. Three of the five best preserved specimens showed perfectly definite points of rup-

TABLE I

The numbers of egg depositions observed in the laboratory are listed according to the dates and hours of occurrence. The hours of observation (—) on the respective dates are indicated.

Date	A.M.							P.M.												Total
	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	
8/9	—	—	—	—	—					—	—	—	—	—	—	—	2			2
10						—	—	—						—	4	3	1	—		8
11						—	—	—						1	—	—	—	—	—	1
12						—	—	—		—	—	—	2			—	—	—	—	2
17						—	—	—		1	—			—	—					1
19						1	—			—	—				—	—				1
23						1	—			—	—				1					2
24	—	—	—						—	—	—	—	1							1
26						—	—	—	—	—	2	—								2
27							—	—		—	—	1								1
Total						2				1	2	1	3	1	5	3	3			21

ture of the ventral integument in the region of the bursa, with the eggs in such a position as to indicate their emergence at this point.

The best two cases are illustrated by Figs. 4 and 5. In Fig. 4, the section passes through the flattened vagina (*v*), the female aperture (♀), the ventral protuberance in which two of the mature eggs appear, and

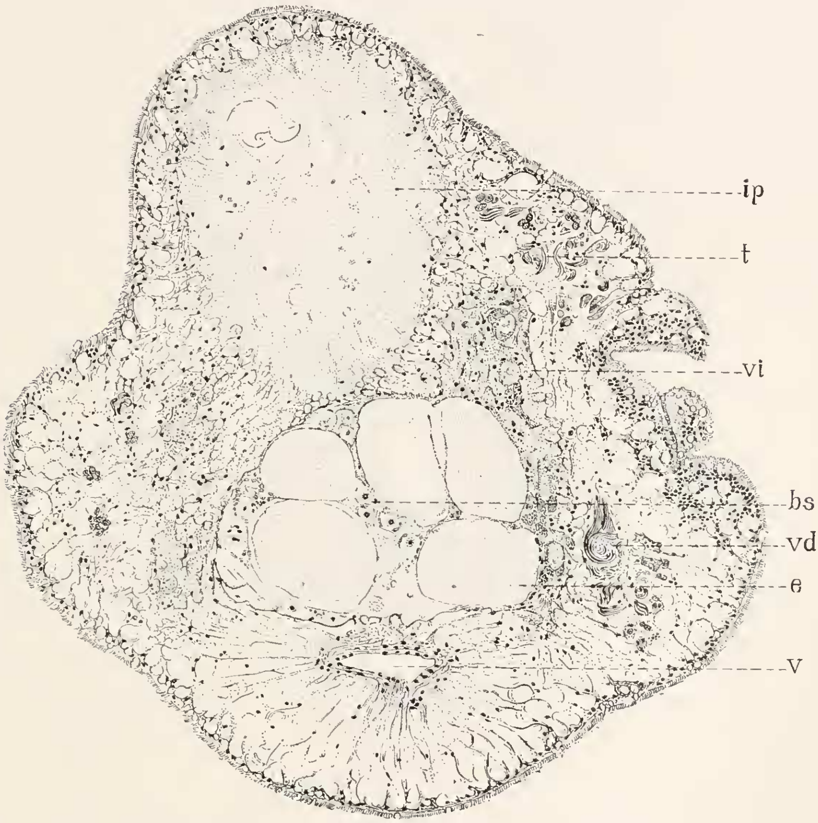
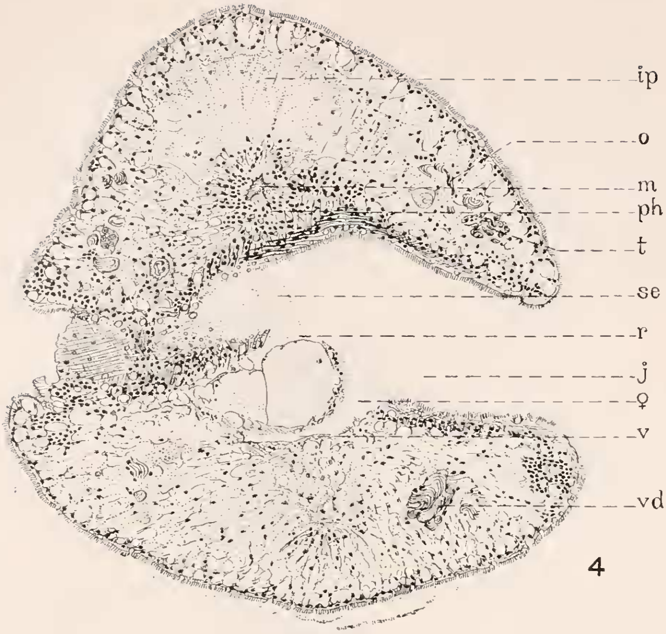


FIG. 3. Oblique frontal section of an egg-laying individual, to show the median ventral position of the mature eggs (*e*), and the displacement of the bursa (*bs*) around the eggs. Hot Heath fixation, stained in Heidenhain's haematoxylin. Magnification 72 ×. *ip*, intestinal parenchyma; *t*, testis follicles; *v*, vagina; *vd*, vas deferens; *vi*, vitellarium.

through the undisturbed mouth (*m*) and short pharynx (*ph*). The ventral integument shows a rupture (*r*) about 50 microns in width near one of the shed eggs (*se*). It is medial, extending through seven consecutive sections (70 microns). A jelly layer (*j*) surrounds the shed eggs and is connected with the ventral surface of the body near the point of rupture. The unshed mature eggs converge toward the point of



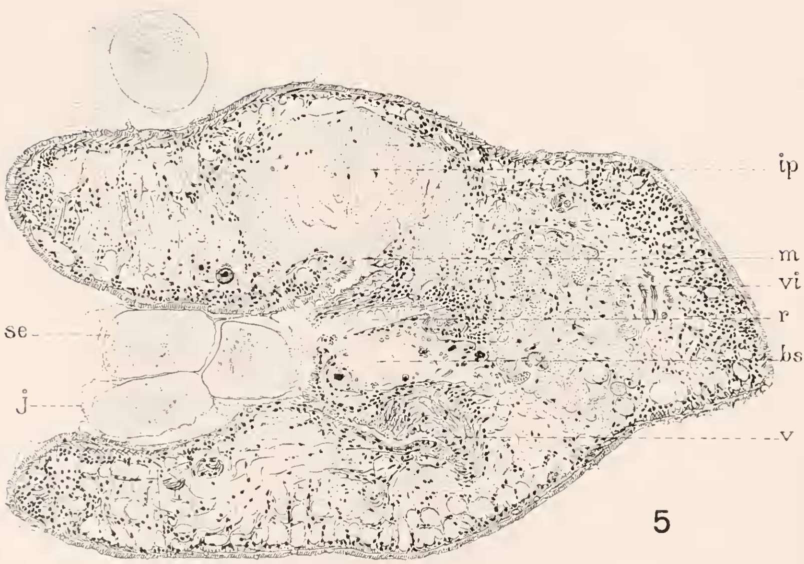


Fig. 4. Same individual as Fig. 3; twentieth section (0.20 mm.) ventral to that of Fig. 3. To show the rupture of the ventral integument (*r*), and the jelly strand connecting one of the two already shed eggs (*se*) with this point. The section passes obliquely frontally through the mid-region and nearly transversely through the mouth (*m*) and pharynx (*ph*). Magnification 50 ×. ♀, female genital aperture; *o*, immature egg; other abbreviations as above.

Fig. 5. Oblique sagittal section of an individual fixed after the extrusion of all of its eggs. To show the broken ventral integument and disrupted bursa, also the undisturbed mouth and intestinal parenchyma, and the flattened vagina. Hot Heath fixation, stained in Heidenhain's haematoxlylin. Magnification 62 ×. Abbreviations as above.

rupture, as shown by the sections of the same individual represented in Figs. 3 and 4. The second specimen, represented in Fig. 5, shows the relation of the bursa, ruptured integument and shed eggs. In this case also the condition of the mouth and the extremely flattened vagina present striking evidence that these structures are not concerned in egg deposition. The ventral integument in the region of the bursa is disturbed and lacks cilia. Bits of tissue and jelly extend from the last shed egg to the region of the rupture. The bursa is greatly vacuolated, a portion of its tissue extending to, and apparently partially closing, the rupture. The disruption of the bursa results indubitably from the pressure of the eggs in their outward passage, combined with the violent muscular contractions observable in this region throughout the egg-laying process.

TABLE II

Distribution of the numbers of eggs per egg mass deposited in fingerbowls by animals collected 8/20/37, from the third to eighth day in the laboratory.

	Numbers of Eggs per Egg Mass					Total Numbers of Egg Masses
	1–5	6–10	11–15	16–20	21–25	
8/23.......	23	72	27	3	1	126
8/24.......	3	4	3	0	0	10
8/26*.......	14	34	19	2	0	69
8/27.......	3	13	19	5	0	40
8/28.......	5	18	12	2	0	37
Totals......	48	141	80	12	1	282

* Two-day interval.

In the remaining seven specimens the exact point of rupture was not clear as a result of either the plane of sectioning, or of distortion following slow fixation. However, they correspond, in all essential features, with the two cases described above.

If *Polychoerus carmelensis* regularly discharges its eggs through rupture of the body wall, evidence of this should be observable in mature specimens fixed at random. Histological examination has shown frequent irregularities in the epidermis, parenchyma and bursa wall, including, in some cases, the interruption of epidermal ciliation. The general appearance suggests regeneration following recent disruption.

A few miscellaneous observations regarding the breeding habits of *Polychoerus carmelensis* in the laboratory and in the tide-pools may be here recorded. Animals brought into the laboratory and placed in fingerbowls do not, as a rule, begin to deposit eggs (or continue egg deposition) until the third day thereafter. During the following three days egg clusters are deposited in abundance, after which they become less numerous. The individual worms do not usually deposit all of their eggs at once, as has been suggested for the closely related *Polychoerus caudatus*. This is attested by the fact that the number of egg masses obtainable from a single dish may exceed the number of worms present. Data relating to the numbers of eggs per egg mass are given in Table II. The figures represent the numbers of egg masses deposited in five fingerbowls during the first eight days in the laboratory.

There appears to be a definite breeding season for this species in the Monterey region. Collections in 1937 were made at intervals from June 22 through July and August. In June no egg masses or young worms were found in the tide pools. By July 8 they were found in abundance on pebbles and shells and in the folds of the *Ulva*. Egg laying continued in the tide pools and aquaria through July and August, although in August there occurred an increasing number of apparently spent, large-sized individuals with reproductive system partially or completely absent. Thus, in 1937, the breeding season began in late June or early July and declined toward the end of August. Less extensive observations indicated that the same situation obtained during the season of 1936.

Discussion

The foregoing account of egg laying in *Polychoerus carmelensis* provides evidence in support of the hypothesis of von Graff and Bresslau that the eggs of some of the Acoela may be discharged through breaks in the body wall. *Convoluta roscoffensis* is the only other species of this group in which egg laying has been described as involving rupture of the parent worm. In that species, however, the body of the worm is frequently broken completely in two at the time of egg deposition. The regenerative powers of the Acoela have been demonstrated by the experiments of Stevens and Boring (1905) and of Child (1907) for the Pacific Coast *Polychoerus,* and by Keil (1929) for the Atlantic Coast *Polychoerus caudatus*. The healing of injured surfaces occurs remarkably rapidly in *Polychoerus carmelensis*. For example, when worms are cut from the posterior end almost the entire length of the body, the two almost separate halves are quickly brought into contact by their normal locomotor activities, and heal within about ten minutes. A small notch may remain at the posterior end, but frequently all trace of the cut is obliterated. For organisms which heal and regenerate so readily the emergence of eggs through rupture of the body wall, in the absence of oviducts, may be regarded as a natural and direct method.

Summary

The egg-laying habits of *Polychoerus carmelensis* are described, with especial emphasis upon the path of emergence of the eggs.

The mature eggs pass from the two vitellaria ventrally and medially between the ventral integument and the bursa seminalis, and emerge to the exterior through a median rupture at the level of the bursa.

The jelly mass is secreted by the entire surface of the animal during repeated rotation, head over tail. The eggs emerge during rotation, probably aided by violent muscular contractions observable at this time.

The entire process of egg laying requires five or six minutes.

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